The smell of the sea

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The smell of the sea is caused by the volatile organic sulphur compound dimethylsulphide (DMS). Low concentrations of DMS in the water (low nM range) and the atmosphere can be found in all marine areas. High concentrations (high nM range) often coincide with blooms of specific marine phytoplankton species. Locally, high concentrations are also observed in the water overflowing beds of specific macroalgae and corals, and in sediments of mudflats and microbial mats.

Since the discovery of DMS as an important volatile sulphur compound, research on its emission from the oceans into the atmosphere has long been the domain of marine and atmospheric chemists. It is now well established that DMS is one of the main natural volatile organic sulphur compounds. Estimates of global DMS emissions vary between 0.5×10^{12} mol S year^{-1} (BATES *et al.* 1987b) and 1.2×10^{12} mol S year^{-1} (ANDREAE 1990). Approximately 90% of the global DMS flux is emitted from the oceans. DMS makes up 50 to 60% of the total natural sulphur flux to the atmosphere. Interest in DMS was not only fuelled by the realisation that marine DMS emissions could close the global sulphur budget, but also because DMS was thought to be involved in the biological regulation of climate (CHARLSON *et al.* 1987). This was hypothesised because oxidation products of atmospheric DMS may act as condensation nuclei, thereby affecting the radiative properties of skies and clouds, with subsequent implications for climate.

The oceans, bearing in them the many biological processes which together determine the DMS concentrations, have long been regarded as a black box. Consequently, quantitative knowledge about these processes is still in its infancy. DMS is derived from the enzymatic cleavage of dimethylsulphoniopropionate (DMSP), which is a compound produced by algae. The cleavage of DMSP is thought to occur mainly after its release from the algal cell. This release is promoted either by cell lysis at the end of a bloom (NGUYEN *et al.* 1988), or by grazing of zooplankton (DACEY & WAKEHAM 1986). Whether or not the actual cleavage reaction is carried out by bacteria, grazers or the algae themselves is still unclear, and will probably vary according to the community structure. Since the last six years, there is increasing evidence that in many marine areas the DMS that ultimately reaches the atmosphere may account for only a small percentage of the DMSP-sulphur originally produced by the algae. An alternative pathway for DMSP conversion is demethylation by bacteria (TAYLOR & GILCHRIST 1991). In addition, the DMS that is produced in the cleavage reaction may partly be consumed by bacteria (KIENE & BATES 1990). That a small percentage of the DMSP-sulphur results in such high fluxes of DMS indicates that only minor changes in the complicated biological processes affecting the DMS concentrations in the water are needed to alter this percentage, resulting in major changes of the flux of DMS to the atmosphere. This stresses the need to investigate the different processes involved.

The role of algae in the marine sulphur cycle has long been thought to be restricted to the production of DMSP, whereas the conversion of this compound was thought to be carried out by bacteria. At the start of this PhD project only

two early papers had been published, which reported on the enzymatic cleavage of DMSP into DMS and acrylate in crude extracts of a macroalga (CANTONI & ANDERSON 1956) and a heterotrophic dinoflagellate (ISHIDA 1968). The release of small amounts of DMS in axenically grown cultures of a Prymnesiophyte had also been reported (VAIRAVAMURTHY *et al.* 1985). Whether or not these algae were able to contribute significantly to the *in vivo* conversion rates of dissolved DMSP into DMS was, however, unknown. Knowledge on the algal contribution is of interest because bacterial biomass is often low during the growth phase of algal blooms; an observation which is especially documented for blooms of *Phaeocystis* sp., the research object of this thesis. If the algal cleavage of dissolved DMSP is significant and bacterial activity is low, then a greater fraction of the DMSP sulphur could escape to the atmosphere, because (1) bacterial demethylation of DMSP will not compete with the DMS production pathway, resulting in more DMS, and (2) there will be little or no biological DMS consumption.

In Chapter 2, *in vivo* experiments with axenically grown *Phaeocystis* sp. are described in which for the first time the enzymatic conversion of dissolved DMSP into DMS and acrylate by algal cells was observed. A culture in the exponential growth phase displayed Michaelis-Menten type kinetics for DMSP conversion, yielding an apparent K_m value for DMSP of 11.7 ± 3.1 µM and a V_{max} value of 3.05 ± 0.48 nmol DMS min^{-1} $(10^6$ cells$)^{-1}$. Dissolved DMSP conversion rates declined during the transition from exponential to stationary growth phase, at least partly due to a diminished overall affinity of the enzyme system(s) involved in DMSP conversion. On the basis of the conversion reaction the enzyme involved was tentatively named DMSP-lyase.

Whether or not this enzyme activity was specific for *Phaeocystis* sp. was subsequently investigated (Chapter 3). An enzyme assay was developed to measure DMSP-lyase activity in extracts of natural plankton assemblages. This assay was used during the spring bloom of 1993 off the Dutch coast in order to test the phytoplankton samples for their DMSP-lyase activity in relation to species composition. The survey was done at an early stage of the bloom when there was a shift along the coast from a diatom-dominated to a *Phaeocystis*-dominated phytoplankton population. A highly significant correlation was observed between *Phaeocystis* sp. abundance and DMSP-lyase activity (r^2 = 0.9660, n = 23), whereas no correlation existed with any of the other species, total diatom numbers, total diatom biomass or total protein content.

In order to get an indication of the potential DMS-production rates by *Phaeocystis* sp. during a spring bloom, calculations were performed based on the *in vivo* DMS-production rate as measured in *Phaeocystis* sp. cultures at the *in situ* sea water temperature. Using a mixed water column depth of 5 m, the calculated DMS-production rate in nearshore waters ranged from 47 to 131 µmol DMS m^{-2} d^{-1} with *Phaeocystis* sp. cell numbers ranging between 8 to 26 × 10^6 cells l^{-1}. Compared with abiotic loss factors for DMS, such as exchange with the atmosphere and photochemical oxidation, these production rates are 1.5 to 4.5 times higher. The strong correlation of the DMSP-lyase activity with

Phaeocystis sp. abundance suggests that during the early stages of a bloom *Phaeocystis* sp. is a dominant if not the main DMS producer. Model calculations for the Southern Bight of the North Sea indeed indicate that DMSP-lyase activity by *Phaeocystis* sp. may contribute 50% of the total DMS production during the spring bloom (VAN DEN BERG *et al.* 1996). This potentially large contribution of *Phaeocystis*' DMSP-lyase and the usually low bacterial numbers during *Phaeocystis* sp. blooms (VAN BOEKEL *et al.* 1992, THINGSTAD & BILLEN 1994), may result in large DMS fluxes to the atmosphere. Many field measurements have shown that high concentrations of DMS coincide with blooms of *Phaeocystis* (BARNARD *et al.* 1984, GIBSON *et al.* 1990, LISS *et al.* 1994).

A greater understanding of the DMSP-lyase activity by *Phaeocystis* sp. was obtained by studying some characteristic properties of the enzyme and by identifying its location within or on the cell. This is discussed in Chapters 4 and 5. Experiments with crude cell extracts provided strong evidence that the enzyme was membrane-bound (50 to 80% of total activity was present in the membrane fraction) and located extracellularly. Association with an intracellular membrane or an internal location on the plasmalemma appeared unlikely considering the already high intracellular DMSP concentrations (ca. 150 mM) and the observed instantaneous conversion of extracellular DMSP by whole cells, even in the low micromolar ranges. Although it is assumed that the enzyme produced by *Phaeocystis* is a DMSP-lyase, the possibility that the conversion of dissolved DMSP is a secondary reaction of an enzyme not specific for DMSP cannot be excluded. Isolation, purification and characterisation of the enzyme may give an answer to this question. Attempts to isolate the enzyme from *Phaeocystis* cells have, however, failed up to now, due to severe losses of activity during the isolation procedure.

With the knowledge that *Phaeocystis*' DMSP-lyase is located extracellularly, the question remained what its physiological role is: whether or not it is used in an active regulation of the internal DMSP concentration, and which conditions trigger such a regulation. For answering this question it is a necessity to have insight in the physiological role of DMSP itself. DMSP is a compatible organic solute, and involved in the osmotic protection of marine algae (discussed in Chapter 7). In experiments with salinity variations a slow adaptation of the DMSP production in *Phaeocystis* sp. cells was observed, affecting intracellular DMSP concentrations on a long term (Chapter 7). Upon salinity shocks, however, short term regulation of the DMSP content in *Phaeocystis* cells was not observed (Chapter 5), nor did salinity shocks effectively alter DMSP-lyase activity (Chapter 4). These results suggest that the production or conversion of DMSP is not directly triggered by salinity, but merely reflects the physiological condition of the cell.

Effects of the physiological condition of algae on the intracellular DMSP content is a variable of which little is known. Other parameters than salinity which appear to have an effect are temperature (KARSTEN *et al.* 1996), light (KARSTEN *et al.* 1992) and the nitrogen status of the growth medium (GRÖNE & KIRST 1992). The actual mechanisms behind these effects are, however,

unknown. It has been suggested that deficiency of nitrogen may result in an increased intracellular DMSP content, as DMSP may substitute for nitrogen-containing organic solutes, thereby saving on the cell's nitrogen demand. Effects of the iron status of the growth medium – another important growth limiting nutrient in vast areas of the world's oceans – had not been studied before. Iron is essential for phytoplankton growth as it controls photosynthesis as well as many enzymatic processes. Effects of iron and light limitation on DMSP production in cultures of an Antarctic *Phaeocystis* strain are presented in Chapter 6. Effects of iron limitation may be reflected in a reduction of the energy supply or in a reduction of the nitrogen assimilation. If the cells would primarily suffer from a reduced energy supply, a reduction of the energy demanding DMSP production was expected. If iron limitation would induce a nitrogen deficiency, an increased DMSP content could be expected, as is the case for true nitrogen-limited conditions. In our experiments, iron deficiency led to strongly reduced cell volumina and growth rates. The light conditions of the cultures appeared to dictate the appearance of iron limitation. We suggest that under low-light and iron-limited conditions a strong reduction in energy supply suppressed photosynthetic carbon fixation more than nitrogen assimilation, resulting in an overall reduction of the cell's metabolism. Cellular DMSP content also became reduced, which was consistent with the observed reduction in cell volume, thereby maintaining cellular DMSP concentrations comparable to the iron sufficient cultures. Under high-light and iron-limited conditions, reducing power is enhanced, and carbon fixation increases relative to nitrate assimilation. Therefore, nitrate assimilation may have been the rate limiting factor for cell metabolism, resulting in increased intracellular DMSP concentrations.

An elaboration of the physiological aspects of DMSP production and conversion is given in Chapter 7, in which an overview is presented of the current knowledge on the biosynthesis of DMSP, possible regulatory mechanisms involved, and suggested metabolic functions of DMSP and its enzymatic cleavage. Compared to other (more) compatible solutes like polyols, DMSP synthesis is an energy-expensive process. During the growth season, however, energy as such is usually not a growth limiting factor, but rather the requirement of nutrients such as nitrogen and iron. It is argued that DMSP production can be regarded as an overflow mechanism coupled to cysteine and methionine metabolism, or as a means of dissipating excess reducing power, bringing nitrogen back into the system, while at the same time saving on the cell's nitrogen requirement. This hypothesis may explain both the high DMSP concentrations in the high-light and iron-limited cultures discussed before, and a phenomenon observed in the first experiments we performed with *Phaeocystis* sp. (Chapter 2). In a senescent culture, the total of DMS and DMSP slightly increased, indicating that even in declining populations of *Phaeocystis* DMSP production can occur. This has also been observed for the production of sugars (Janse *et al.* 1996), and may be regarded as a means of dissipating energy under nutrient limited conditions.

Considering again the physiological function of DMSP-lyase, it can be concluded that this enzyme is not involved in the short term osmotic adaptation of the cell. If the production of DMSP is indeed coupled to cysteine and methionine metabolism, then the equilibrium concentration of DMSP must be regulated by its degradation rather than by its production. One way of doing this is by transporting DMSP out of the cell. Subsequent removal of DMSP by extracellular cleavage into DMS and acrylate will facilitate this release, as DMSP concentration gradients across the membrane are kept maximal. This is especially relevant for *Phaeocystis* sp., as the mucus layer surrounding colonial cells will increase the diffusive boundary layer around these cells. In addition to the maintenance of a DMSP gradient, the cell may simultaneously profit from the release of acrylate and protons upon cleavage. The released protons can be used for e.g. nutrient uptake. A possible build-up of acrylate within the microzone may result in concentrations and conditions with antibacterial activity (SIEBURTH 1960), as opposed to the more dilute conditions in seawater, where acrylate may serve as a substrate for bacteria. In this way, the otherwise wasteful release of DMSP can be of some benefit to the cell. Whether this sufficiently explains the maintenance of a DMSP-specific lyase remains to be investigated.

This thesis provides evidence that *Phaeocystis* sp. plays an important role in the production of DMS, which is largely the consequence of its ability to convert dissolved DMSP into DMS enzymatically. Due to its ubiquitous distribution and its observed dominance during spring blooms, *Phaeocystis* sp. also appears to be of importance in other elemental cycles. With regard to climate change studies, the sulphur as well as the carbon cycle is of particular relevance. *Phaeocystis* sp. blooms may form a sink for CO_2 due to increased sedimentation of carbon enriched material. In this respect, the observed variability of the *Phaeocystis* sp. bloom structure, when comparing different geographical areas, represents a potential for both shifts in DMS emissions to the atmosphere and for variability in the biological carbon pump. In order to get a greater understanding of its global significance, *Phaeocystis* deserves further research.